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Effects of Aqueous and Ethanol Extracts of Cassia alata on Yam Rot

Aji, P.O.[1] Tunwari, B.A.[2] Kyugah J.T.[1]
1.Department of Biological Science, Federal University Wukari, Katsina - Ala Road, P.M.B. 1020, Wukari, Taraba State, Nigeria
2.Department of Crop Production and Protection, Federal University Wukari, Katsina - Ala Road, P.M.B. 1020, Wukari, Taraba State, Nigeria

Abstract
The objective of this study is to isolate, identify and to determine the effect of various concentrations of aqueous and ethanol extracts from the leaves of the test plant *Cassia alata* in the control of fungal isolates from yam rots in Wukari Nigeria. Rotted yam tubers were obtained from three markets in Wukari. The ingredients of test plant were extracted by aqueous and ethanol solvents. Leaf extracts of different concentrations (0 %, 20 %, 40 % and 60 %) of aqueous and ethanol extraction of the test plant was poisoned to growth media prior to inoculation. The fungi associated with the spoilage of the sample of the yam tuber were identified base on their morphological characteristics. Of all the samples studied, three species of fungi were found to be associated with the yam rots. The most commonly isolated fungi were *Aspergillus niger* and others are *Aspergillus flavus* and *Rhizopus stolonifer*. All concentrations used suppressed the mycelia growth of the tested pathogens. The effect was proportional to concentration and inhibition value was lowest at 60 % concentration, For aqueous and ethanol extractions, *Cassia alata* was more effective on Aspergillus niger, Phytochemical analysis showed that the presence of alkaloids, flavoniods, tannin,s saponins, phenols, glycosides, terpenoids, anthracenes and steroids in the leaves of *Cassia alata*, might have conferred the anti-fungal properties on these fungi species. Application of the fungal pathogens on the fungal pathogens isolated on healthy tubers and the subsequent development of rots confirmed these organisms as the natural pathogens of this crop. The extracts from both aqueous and ethanol exerted significant anti-fungal effect on all the test organisms at 60 % concentration, and this concentration would help to minimize infection and spoilage during and after storage and improve farmer' revenue.
Keywords: Yam rot, leaf extracts, *Acasia alata*, pathogenicity, phytochemical

1. Introduction

Yam *(Dioscorea spp)* constitutes an economically important staple for millions of people in the tropics and sub tropics. West Africa account for about 95% of world production and 93% of the total yam production area (FAO, 2012). Out of the world production of over 30 million metric tons per annum, Nigeria alone produces 22 million tons annually. Nigeria is the World's largest producer of yam (Dioscoreacea; *Dioscorea spp.*) producing 35.017 million MT of the tuber annually (Kleit *et al.,*2012) .Postharvest harvest deterioration or rot caused by various microorganisms is seen as the single most important factor militating against commercial yam production in Nigeria besides lack of research for development and capacity building in yam base research for development and capacity building in yam base researches (Taiga, 2012; Onyeka, *et al*., 2011). Rots according to Taiga (2011) result in 7 million MT of yams annually. Rot of yam tuber may be soft, wet or dry and could occur pre – or post –harvest. Pre – harvest rots are due to infection of tuber by soil-borne pathogens.

Most rot of yam tubers are cause by pathogenic fungi such as *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae,Fusarium oxysporium, fusarium solani, Penicillium chrysogenum, Rhizoctonia spp, Penicillium chrysogenum, Rhizoctonia spp.,Penicillium oxalicum,Trichoderma viride* and *Rhizopus nodosus (*Ezelbekwe and Ibe, 2010; Twumasi *et al*, 2014). A number of treatments and techniques have been developed to reduce these physiological activities and also protect the tuber from postharvest diseases. These include treatment with chemicals, plant extractsts, palm wine and gamma irradiation; storage techniques used include cold storage, improved underground storage and improved yam barns (Osunde, 2008)

Yam tuber may be harvested 6-10 months after emergence depending on species. Harvesting is usually done by carefully digging the tubers out of the soil with the aid of stout pegs, cutlasses or hoes. The yield depends on the size of the seed piece, species and environment but normally ranges from 8-50 t/ha in 6-10 months. West Arica accounts for 90 - 95 % 0f world yam production with Nigeria being the largest single producer (Kathryn *et al.,* 2012).

Yams may also contain small quantities of polyphenolic compounds (e.g tannins), Alkaloid and steroid derivatives. The carbohydrate content of tuber represents its major dry matter component and may be classified as starch, non-starch, polysaccharides and sugar (Babuand and Parimalavalli, 2012).

One of the most pressing problems facing the countries of the third world is food scarcity. It is reported that nearly 1 billion people are challenged by severe hunger in these nations of which 10 % actually die from hunger –related complications. A substantial part of this hunger problem stems from inadequate agricultural storage and

produce preservation from microbes-induced spoilages (Salami and Popoola, 2007; Kana *et al*, 2012). According to Arya (2010), of all losses caused by plant diseases, those that occur after harvest are the most costly. Cassava, yam and sweet potato are important source of food in the topic. Generally rotten start from the field and progress in storage .postharvest fungi rot are up to 10% in yam (Ikotun, 1986). This may aggravate to 50% in some instances (Okigbo, 2005; Okigbo *et al,* 2009) especially now given the challenge of climate change (sadiku and sadiu, 2011). The research paper presents the research findings on the effect of aqueous and ethanolic extracts of *Cassia alata* leaves and their concentrations for *in vitro* control of yam rot.

2. Materials and Methods

2.1 Materials used for isolation of pathogens

Potato dextrose agar, Distilled water, Mercury chloride, Vaseline, Cotton wool, Aluminium foil paper, Ethanol, What man Filter Paper, Cheese cloth, Conical flask, Petri dish, Cork borer, Inoculating needle, Rotted yam tuber.

2.2 Collection of yam

Yams with symptoms of rot were obtained from New Market and Old Market. The yams were being identified as being rotted by comparing with fresh healthy ones.

2.3 Collection of Plant materials, preparation and determination of the plant extract concentration

Leaves of *Cassia alata* (Plate 1) was obtained from and within Taraba State University and identified in crop production and protection department, Federal university wukari, Taraba State. The method of Ijato (2011) was used to prepare both aqueous and ethanolic extracts. The plants were taken to the Biological Science Department, Federal University Wukari, Taraba State. The collected plant leaves were washed thoroughly under running tap water and allowed to air dry for 7 days. These were then grinded into fine powder. After that 20 g, 40 g and 60 g of the powder materials were each added to 100 mIs of distilled water in separate conical flasks respectively. This were vigorously shaken and left to stand for 24 hours. The samples were filtered with 3 layers cheese cloth to obtain the 20 %, 40 % and 60 % concentrations of the aqueous extract. The same procedure was used for obtaining 20 %, 40 % and 60 % ethanol extract.

2.4. Pathogenicity Test

Pathogenicity test was carried out using techniques of Okigbo *et al.* (2009). Healthy yam tuber was washed with sterile distilled water, wiped dry using Whatman No.1 filter paper and surface sterilized with 0.1% mercury chloride solution to remove surface contaminants and rinsed in three changes of sterile distilled water. A sterile 2 mm cork borer was used to make a 2 mm cut on the yam tuber and then culture of the isolates were inoculated into the open cut surface and the removed tissue was replaced and sealed with vaseline jelly. Yam tuber was inoculated in three replicates. The yam tuber was incubated for 14 days. On establishment of disease symptoms, the infected yam tissue was taken and cultured until pure cultures were obtained. The morphological and microscopic characteristics of the Isolates were compared with the original isolate.

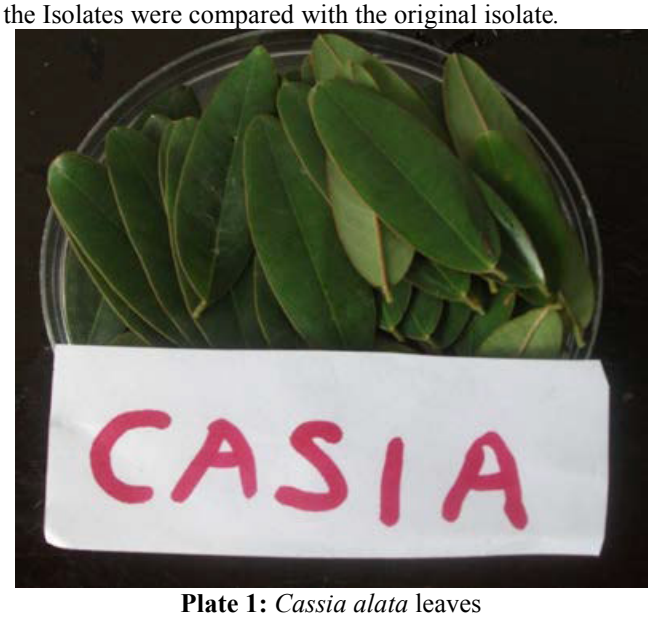

Plate 1: *Cassia alata* leaves

2.5. Effect of Plant Extracts on Fungal Mycelia Growth

The approach of Ijato (2011) was used to evaluate the effect of the extract on fungal growth by creating four

equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extract was poured into the flask, plugged with cotton wool and wrapped with aluminum foil to avoid contamination (Madari and Singh, 2005). About 2 mls each of different concentrations of extract of *Cassia alata* was separately introduced into the Petri-dishes containing the media (poisoned food method). Control experiments were without addition of any plant extract but sterile distilled water. Fungitoxicity was determined in terms of percentage colony inhibition % (Nene and Thalpiyal, 2000) using the formulae below.

$$\text{Percentage Colony inhibition} = \frac{DC - DT}{DC} \times 100$$

Where;

DC = Average Diameter of fungal colony in control
DT = Average diameter of fungal colony with treatment.

2.6. Experimental Design and Data Analysis

The research consisted of experiment involving aqueous and ethanolic plant extracts each at 60 %, 40 %, 20 % and 0 % concentrations. The experimental layout was completely randomized design of the aqueous and ethanolic plant extracts experiments. The experiment was replicated three times. All the data was analyzed using analysis of variance (ANOVA) with Genstat 8.1 (2005) statistical package. Duncan's Multiple Range test (DMRT) according to Gomez and Gomez (1984) was used to separate the means where there was significant difference at 5 % level.

3. Result and Discussion

Results of isolation and identification of the causative organisms of the rotted yam, indicated that three fungi were identified namely *Aspergillus niger, Rhizopus stolonifer* and *Aspergillus flavus*. The organisms were earlier found associated with postharvest yam rot (Ogundana *et al.,* 1970; Okigbo, 2002, 2005). Table 1 shows the percentage reduction of mycelia growth of spoilage fungi cultured in PDA incorporated with aqueous and ethanol plant extracts each at 20 %, 40 % and 60 % concentrations. The results showed that aqueous leaf extracts and ethanolic leaf extracts inhibition progressed and more mycelia reduction was recorded at higher concentrations. Oyelana *et al*. (2011) reported that the extracts of Ficus species inhibited the growth of fungi species isolated from rotted yam at 75 – 100 mg ml^{-1} concentrations more than at 25$mgml^{-1}$.

The water and ethanol extracts (Table 1) of the *Casia alata* screened *in vitro* at varied concentrations, showed varying degrees of toxicity to *Aspergillus niger, Rhizopus stolonifer* and *Aspergillus flavus*, expressed as percentage inhibitions to mycelia growth. Ethanol extracts were more effective in reducing the growth of the rot-causing pathogens than water extracts of all the *Casia alata* leaf extracts concentrations tested. The highest growth reduction of the pathogens was recorded at 60 % concentration of the ethanol plant extract. Ethanol extracts exhibited relatively stronger fungitoxicity than water extracts on the test fungi. The differences in fungitoxicity between the extraction medium can be attributed to the difference in the quantity and nature of their active ingredients (Onifade, 2000; Okigbo and Odurukwe, 2009; Nweke, 2015). The results of the phytochemical analysis in Table 2 showed that the leaves of *Casia alata* extracted by both aqueous and ethanol media had strong present of flavonoids. The of flavonoids might been responsible for the reduction of mycelia growth of the pathogens *in vitro* (Oyelana *et al*. 2011).

The pathogenicity test (Table 3) confirmed the natural pathogens responsible for the rot disease in the sampled yam tubers. The intrinsic ability of some exposed yam tubers has equally been reported (Okigbo and Ogbonna, 2006; Oyelana *et al*. 2011). The average spread of the rotted area at 14 days after incubation (4.32 – 6.80 cm) was observed for all the fungal species. *Aspergillus niger* exhibited a wider area (6.80 cm) followed by *Aspergillus flavus* with 5.70 cm spread (Table 3).A significantly different result was reported by Oyelana *et al*. (2011) in which they observed that *Penicillium chrysogenum* exhibited a 62 mm spread and a 60 mm and 55 mm spread by *Fusarium solani* and *Aspergillus flavus* respectively. These implicated organisms posed a significant threat to the revenue of farmers and the health of consumers.

Table 1: Effects of Extracts of *Cassia alata* on the Growth of the Isolates

Concentrations (%)	Solvent	*A. niger*	*R. stolonifer*	*A. flavus*
0	Aqueous	76.33[a]	70.44[a]	77.68[a]
0	Ethanol	78.00[a]	64.22[b]	74.71[a]
20	Aqueous	45.31[b]	54.57[c]	47.18[b]
20	Ethanol	41.43[b]	49.93[c]	42.18[c]
40	Aqueous	41.76[b]	46.74[cd]	39.43[cd]
40	Ethanol	32.15[c]	39.41[e]	29.31[e]
60	Aqueous	28.09[c]	33.19[f]	29.72[e]
60	Ethanol	23.57[cd]	28.33[g]	27.28[e]
Means		43.83	48.31	45.94
S.E.		3.42	3.42	3.93
F - probability		**	**	**

Means in the same column followed by the same superscript(s) are not significantly different (0.05) using Duncan's Multiple Range Test.

Table 2: Quantitive Determination of phytochemical Groups of Extract of Test Plant Leaf

Phytochemical	*Cassia alata*	
	Aqueous	*Ethanol*
Anthraquinones	-	-
Alkaloids	-	-
Flavonoids	++	+++
Glycoside	+	+
Saponins	+	+
Tannins	+	+
Phytobatannins	-	-
Terpenes	-	-
Steroid	-	-
Polyphenol	-	-

Keys: +++ = Strongly Present + = Mildly Present - = Absent

Table 3: Pathogenicity Test of the Pathogens Isolated from Yam Tuber

Days	Mycelia growth of fungal organisms		
	A. niger	*R. Stolonifer*	*A. flavus*
1	0.00	0.00	0.00
2	0.00	0.00	0.00
3	0.00	0.00	0.00
4	1.50	0.00	0.00
5	2.30	0.00	0.85
6	3.60	0.00	1.10
7	4.50	0.00	1.50
8	5.20	0.95	1.87
9	5.95	1.30	2.35
10	6.10	1.97	3.64
11	6.35	2.70	3.97
12	6.50	3.20	4.30
13	6.55	3.70	5.10
14	6.80	4.32	5.70
SE	0.47	0.43	0.46

4. Conclusion

The inhibitory effect of the plant extract could be alluded to the presence of anti-mycelia substances. Greater inhibition of fungal growth was observed at higher concentrations of the aqueous and ethanol extracts. *Aspergillus niger, Rhizopus stolonifer* and *Aspergillus flavus* are common pathogenic fungi which cause tuber rot, fruit and vegetable rot. The results of the investigation are vivid indications for the potential of plant extracts to control fungal pathogens. It is clear from the result that the test plant extract significantly reduce the radial growth of isolated fungi at higher concentration.

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